JCB

Chitale and Richly, https://doi.org/10.1083/jcb.201704028

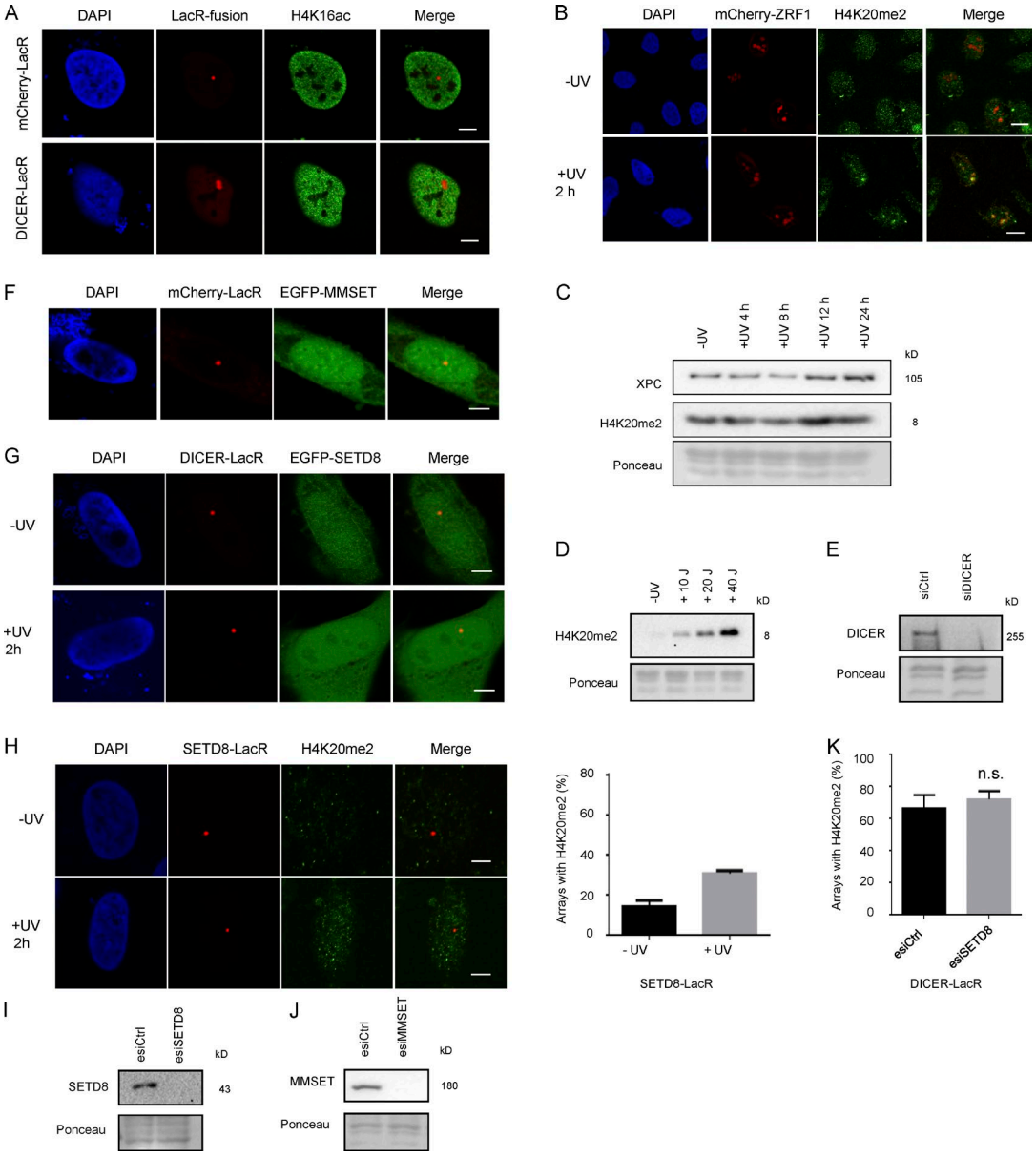

Figure S1. **UV exposure causes formation of H4K20me2 foci that are independent of SETD8. (A)** DICER does not change H4K14ac distribution at chromatin. Immunofluorescence images showing distribution of H4K14ac in U2OS 2–6-3 cells containing a chromatin array tethered with either mCherry-LacR or mCherry-LacR-DICER. Bars, 5 µm. **(B)** UV exposure results in colocalization of H4K20me2 with ZRF1 foci. Immunofluorescence images showing nuclear distribution of H4K20me2 in UV-unexposed and UV-exposed U2OS cells expressing mCherry-ZRF1. Bars, 25 µm. **(C)** There is no global increase of H4K20me2 levels after UV damage. Western blots showing levels of the indicated proteins in whole-cell extracts at various time points after UV exposure. Ponceau is used as a loading control. **(D)** Increasing UV dose leads to an increase in H4K20me2 levels. Western blot showing levels of H4K20me2 in the chromatin fraction 2 h after UV exposure at the indicated dose. Ponceau is used as a loading control. **(E)** Western blot showing the siRNA-mediated DIC ER knockdown. Ponceau is used as a loading control. **(F)** MMSET is not recruited to a control LacO array. Immunofluorescence images showing distribution of EGFP-MMSET in undamaged cells with mCherry-LacR arrays. Bar, 5 µm. **(G)** DICER does not recruit SETD8 to chromatin. Immunofluorescence images showing distribution of EGFP-SETD8 in cells with DICER-LacR–tethered arrays in both UV-unexposed and exposed cells. Mild recruitment was observed in 3 out of 20 cells in both conditions. **(H)** Immunofluorescence images showing H4K20me2 distribution in U2OS 2–6-3 cells, expressing mCherry-LacR-SETD8 (SETD8-LacR; left). The LacO array is visualized by binding of SETD8-LacR. Bars, 5 µm The graph on the right shows a quantification of the number of arrays showing an H4K20me2 mark in unexposed cells (–UV) and 2 h after UV exposure (+UV). Colocalization was measured from three independent experiments, counting 30 cells per experiment. **(I and J)** Western blots showing the esiRNA-mediated knockdowns of the indicated proteins. Ponceau is used as a loading control. **(K)** SETD8 is not required for DICER-dependent setting of H4K20me2. The graph shows a quantification of the number of DIC ER-LacR arrays showing an H4K20me2 mark in cell lines with the indicated esiRNA-mediated knockdowns 2 h after UV exposure (+UV; mean ± SEM). Colocalization was measured from three independent experiments, with 30 cells per experiment.

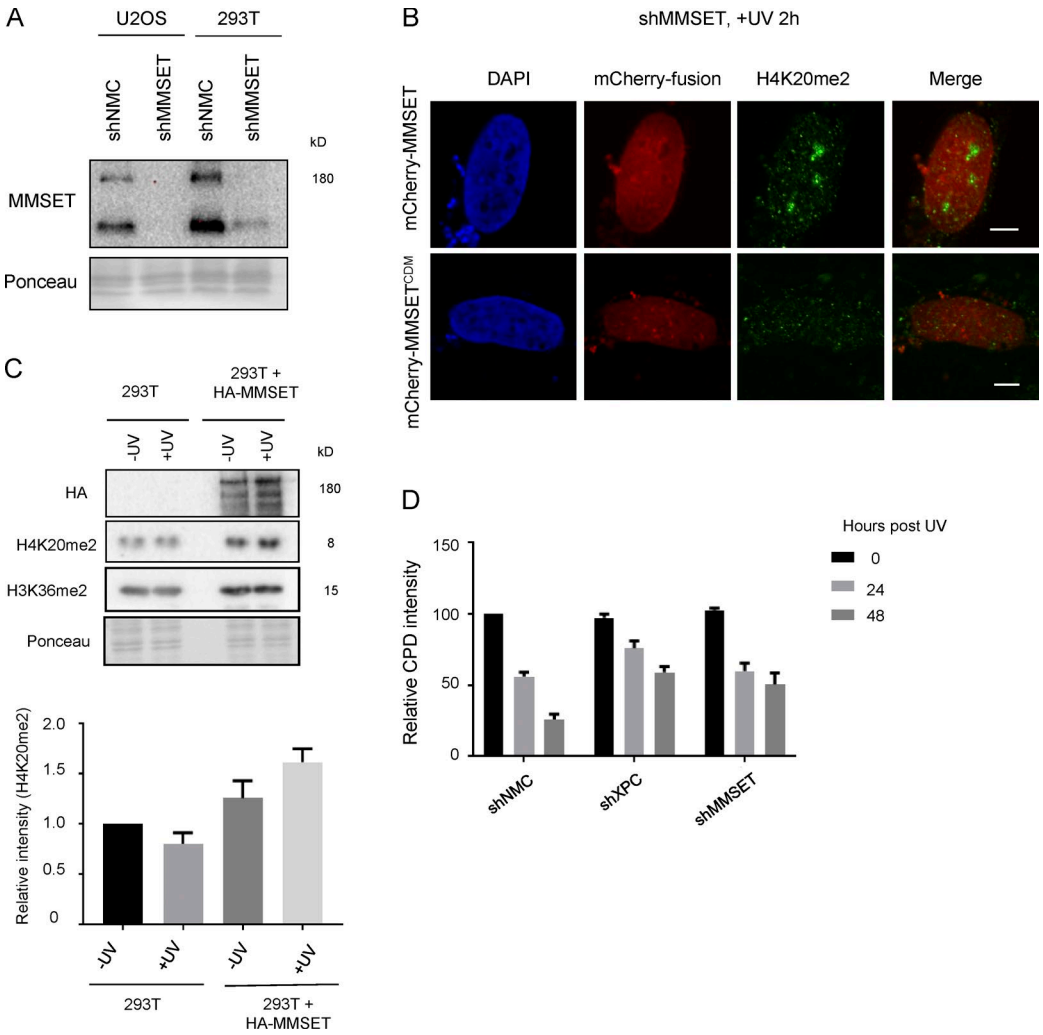

Figure S2. **MMSET interacts with ZRF1 and DICER and is required for CPD removal. (A)** Western blots showing the levels of MMSET in stable shRNA-mediated knockdowns cell lines generated in the indicated cells. Ponceau was used as a loading control. **(B)** MMSET can rescue formation of H4K20me2 foci. Immunofluorescence images showing the distribution of H4K20me2 in shMMSET U2OS cells 2 h after UV exposure. Cells were transfected with either mCherry-MMSET or mCherry-MMSET^{CDM}. Bars, 5 µm. **(C)** Overexpression of MMSET can lead to an increase in H4K20me2 levels. Western blot showing the levels of the indicated proteins in cells transfected with either empty plasmid or HA-MMSET in both UV conditions (top). Ponceau was used as a loading control. The graph on the bottom shows the relative band intensity for H4K20me2 in the indicated conditions. Intensity was measured from three independent experiments. **(D)** The graph shows the relative CPD intensity at various time points after UV exposure in U2OS cells with stable knockdowns of the indicated proteins (mean ± SEM). CPD intensity was measured from three independent experiments, with intensities quantified for 500–1,000 cells per experiment.

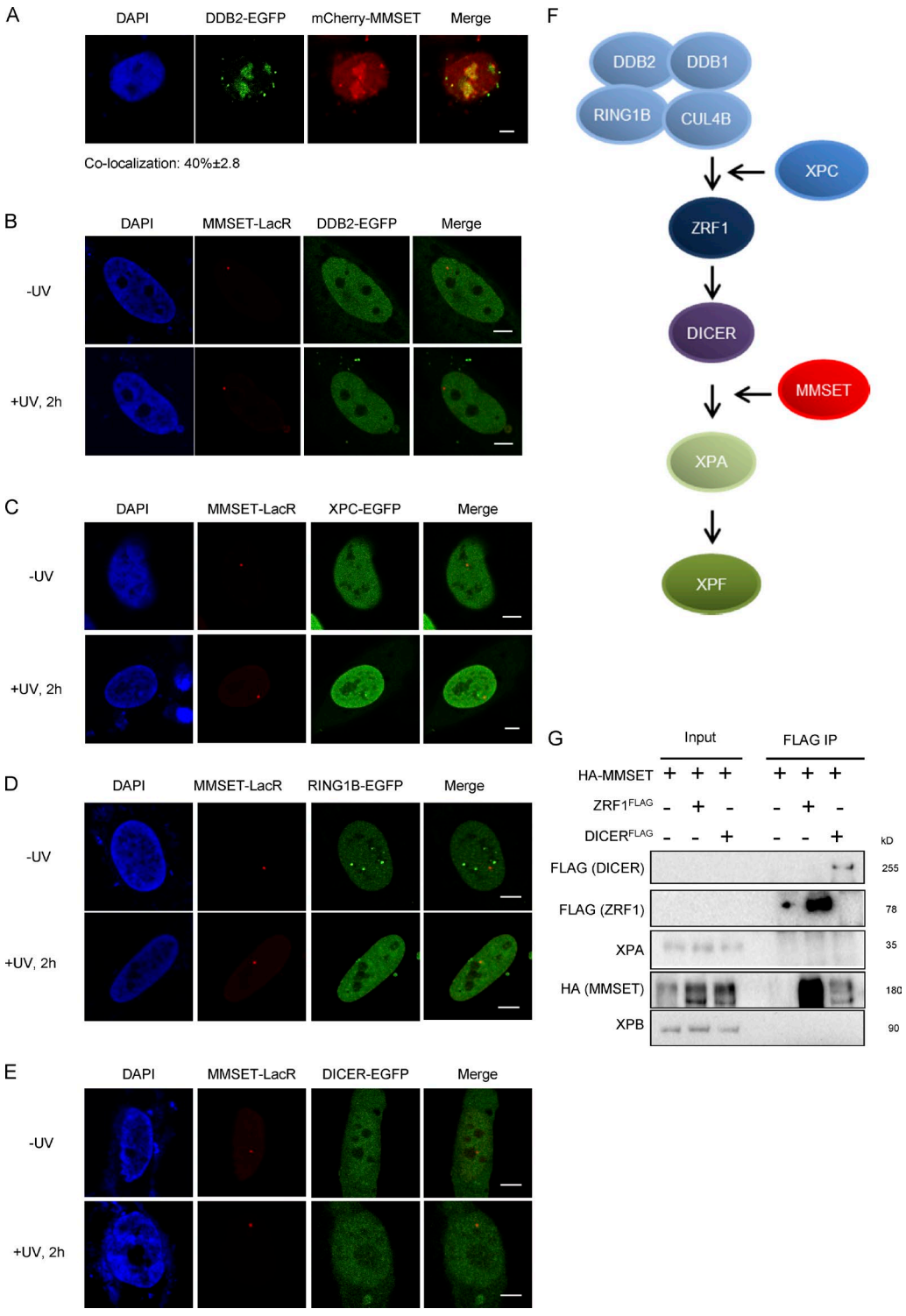

Figure S3. **MMSET is required for NER and interacts with the NER machinery. (A)** MMSET is recruited to sites of UV damage. Immunofluorescence images showing distribution of DDB2-EGFP and mCherry-MMSET in U2OS cells subjected to damage through a 3-μm micropore membrane. The damage sites are marked by DDB2-EGFP. MMSET is observed at ≈40% of lesions 30 min after UV exposure. Bar, 5 μm. **(B)** DDB2 is not recruited to MMSET-LacR–tethered arrays. Immunofluorescence images showing distribution of DDB2-EGFP in cells with MMSET-LacR–tethered arrays in UV-unexposed and exposed cells. No recruitment was seen in 20 out of 20 cells. Bars, 5 μm. **(C)** XPC is not recruited to MMSET-LacR–tethered arrays. Immunofluorescence images showing distribution of XPC-EGFP in cells with MMSET-LacR–tethered arrays in UV unexposed and exposed cells. No recruitment was seen in 20 out of 20 cells. Bars, 5 μm. **(D)** RING1B is not recruited to MMSET-LacR–tethered arrays. Immunofluorescence images showing distribution of RING1B-EGFP in cells with MMSET-LacR–tethered arrays in UV unexposed and exposed cells. No recruitment was seen in 20 out of 20 cells. Bars, 5 μm. **(E)** DICER is not recruited to MMSET-LacR–tethered arrays. Immunofluorescence images showing distribution of DICER-EGFP in cells with MMSET-LacR–tethered arrays in UV-unexposed and exposed cells. No recruitment was seen in 20 out of 20 cells. Bars, 5 μm. **(F)** Schematic showing sequential recruitment of NER factors. The figure shows the hierarchy of recruitment for specific NER factors and indicates a possible position for MMSET in the hierarchy. **(G)** Both DICER and ZRF1 interact with MMSET. Western blot shows levels of select proteins in FLAG purifications performed from cells transfected with the indicated plasmids.

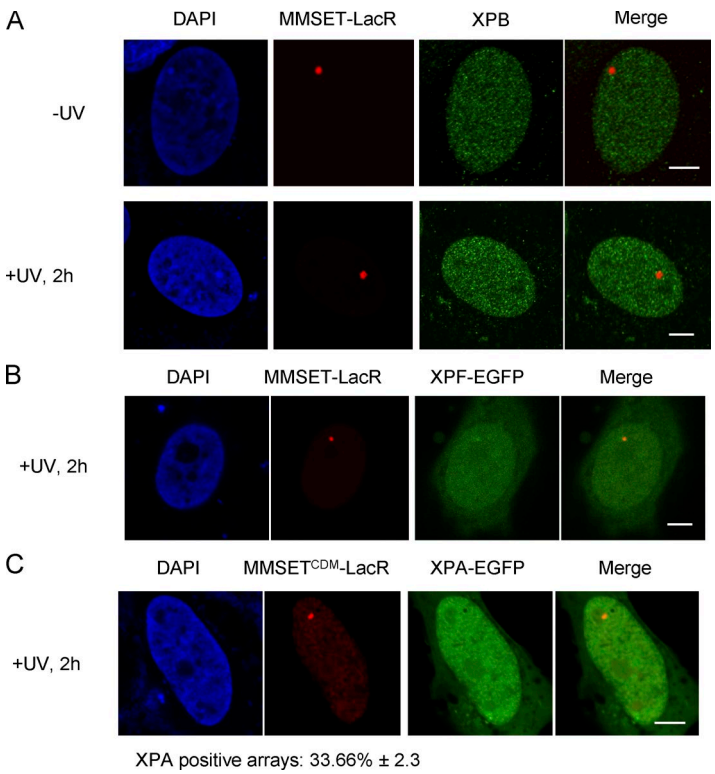

Figure S4. **MMSET-LacR recruits late NER factors to chromatin. (A)** XPB is not recruited to MMSET-LacR–tethered arrays. Immunofluorescence images showing distribution of XPB in cells with MMSET-LacR–tethered arrays, in UV-exposed and unexposed cells. No recruitment was seen in 20 out of 20 cells in both conditions. Bars, 5 µm. **(B)** XPF is weakly recruited to MMSET-LacR–tethered arrays. Immunofluorescence images showing distribution of XPF-EGFP in cells with MMSET-LacR–tethered arrays, in UV exposed cells. Weak recruitment was seen in 15 out of 20 cells. Bar, 5 µm. **(C)** MMSET^{CDM}-LacR is unable to recruit XPA. Immunofluorescence images showing the distribution of XPA-EGFP in cells with MMSET^{CDM}-LacR–tethered arrays 2 h after UV exposure. Bar, 5 µm.

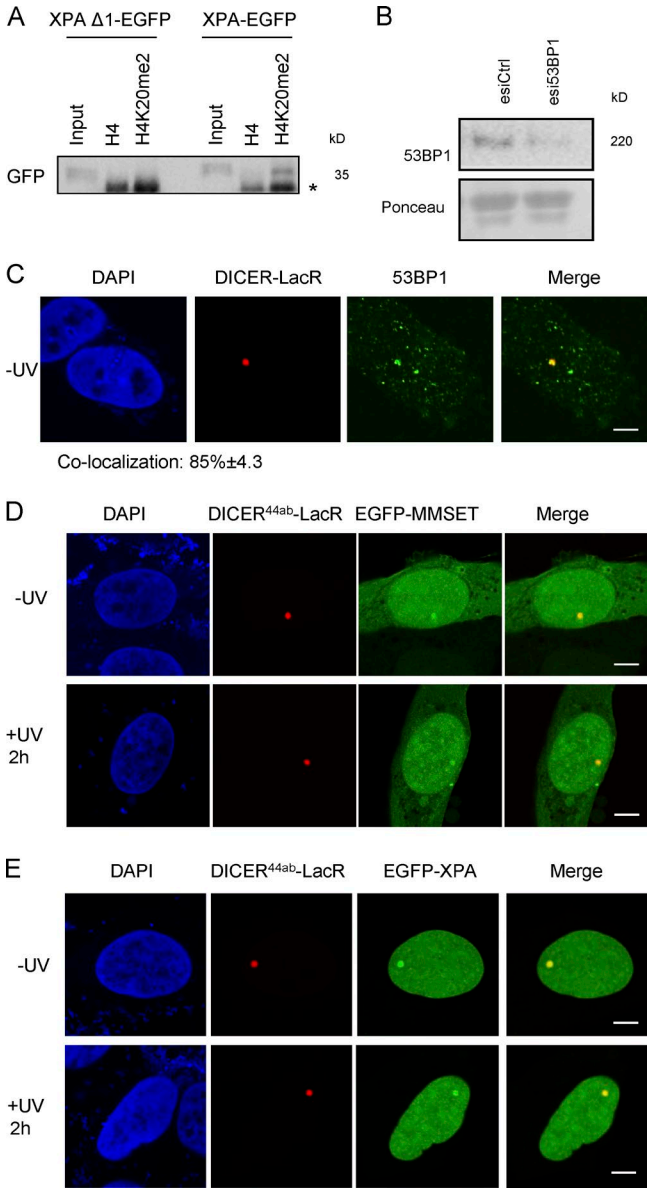

Figure S5. **XPA recruitment to H4K20me2 requires presence of RPA, but is independent of the endoribonuclease activity of DICER. (A)** The RPA32 domain is essential for XPA recruitment to H4K20me2. Pull-downs with H4 and H4K20me2 peptides and protein extracts expressing tagged full-length XPA (XPA-EGFP) or an XPA fusion protein lacking the RPA32 domain (XPA Δ1-EGFP). The asterisk denotes an unspecific band appearing when using the GFP antibody. **(B)** Western blot showing esiRNA mediated 53BP1 knockdown. Ponceau was used as a loading control. **(C)** 53BP1 colocalizes with DICER-LacR–tethered arrays in cells unexposed to UV. Bar, 5 μm. **(D)** DICER^{44ab} can recruit MMSET to chromatin. Immunofluorescence images showing distribution of EGFP-MMSET in cells with DICER^{44ab}-LacR–tethered arrays in both UV-unexposed and exposed cells. Bars, 5 μm. **(E)** DICER^{44ab} can recruit XPA to chromatin. Immunofluorescence images showing distribution of EGFP-XPA in cells with DICER^{44ab}-LacR tethered arrays, in both UV unexposed and exposed cells. Bars, 5 μm.

Provided online are two tables in Excel. Tables S1 and S2 give further information on the plasmids used in this study, as well as the sequences of all si/esiRNAs used in the study.